Addendum #5 to Data Evaluation Record (DER) for MRID 42836101
Aquatic Plant Toxicity test using *Lemna gibba*, Tier I (Guideline 122-2)

Citation: Hughes, J.S. and P.H. Balcom. 1993. The Toxicity of DCPA Technical to *Lemna gibba* G3. Laboratory Project ID No. B038-033-4. Conducted by Malcolm Pirnie, Inc., Tarrytown, NY. Submitted by ISK Biotech Corporation, Mentor, OH. EPA MRID No. 428361-01.

Guideline: 122-2 (Aquatic Plant Toxicity test using *Lemna* spp., Tier I)

Chemical: Chlorthal Dimethyl (DCPA) (PC 078701)

DP Barcode: 386043

Reviewers: Christina Wendel, Biologist, EFED, ERB2 Christina Wendel 4/5/11

Kristina Garber, Biologist, EFED, ERB2 4/5/11

Date: April 5, 2011

Purpose: There were concerns with the solubility of the compound (0.5 ppm), and as a result all aquatic studies were further reviewed to check validity, specifically relating to the measurements of treatment concentrations. In addition, the statistical analysis completed in the original review compared the treatment group(s) to the solvent control only. Therefore, the statistics had to be recalculated comparing the treatment group(s) to the negative control alone.

Method: Statistical analyses were completed using TOXSTAT, as NUTHATCH could not be used, since there was only one treatment group and two controls (negative and solvent). T-tests (in TOXSTAT) were used to determine if there were significant differences between the solvent and negative controls. To estimate the EC_{50} and NOAEC, both Dunnett's and Tukey Test of multiple comparisons were used to compare the means of the treatment groups independently (in TOXSTAT).

Results: The study results originally reported for the *Lemna* aquatic plant toxicity test indicated that the nominal concentration of 11.0 mg/L significantly reduced frond number over a 14-day period, and had 21.4% frond inhibition as compared to the solvent control (see Table 1). The original reviewers also reported that the treatment solution remained cloudy throughout the test, but "was present at its maximum solubility (0.5 mg/L)." The original reviewers determined that the EC_{50} was greater than 11.0 ppm, and the study was classified as acceptable meeting guideline requirements for Tier I non-target aquatic plant study using *Lemna gibba* G3.

The new analysis compared the treatment group to the negative control, as only one test concentration was used; it was represented as a potential limit test.

However, significant differences between the frond counts of the negative and solvent controls were observed at days 7 and 14 (see appendix 1). On day 14, the percent inhibition of the solvent control as compared to the negative control was 13%, which was significantly different (see Table 2). The size of the fronds in the solvent control were noted to be reduced on day 14 of the test. Based on this information, the solvent may have impacted the growth of *Lemna gibba*.

Study Classification: The study is now classified as invalid.

Table 1. *Lemna gibba* reported measurements from the study, and percent inhibition calculation using the solvent control as reported in the original DER for this study.

Nominal Concentration, (mg/L)	Mean Frond Counts on day 14	Percent Inhibition
Solvent Control	647	--
11.0	512	21.4%

Table 2. *Lemna gibba* reported measurements from the study, and recalculated percent inhibition calculation using the negative control.

Nominal Concentration (mg/L)	Mean Frond Counts Day 7	Mean Frond Counts Day 14	Percent Inhibition Day 14
Negative Control	166 (±3)	742 (±34)	--
Solvent Control	149 (±1)	647 (±21)	12.8%
11.0	111 (±4)	512 (±27)	31.5%

(± SD) - Standard deviation

Reviewer Comments: This study was originally reviewed by Michael Davy and Daniel Rieder in 1994. The details of the method of this study are provided in the original DER for this study.

The aquatic plant toxicity study using *Lemna gibba* was originally classified core (*i.e.*, acceptable).

The **aquatic plant toxicity study using *Lemna gibba* is reclassified as invalid** because of the following:

1) The actual concentration that the test organism was exposed to is unknown because:
   - The nominal treatment concentration was 11.0 mg/L. The test concentrations were not measured during the study.
   - At test initiation and throughout the test the treatment solution appeared cloudy with white particulates.
   - The test material was neither centrifuged nor measured.
   - It is likely that the concentration that the test organisms were exposed to was at least the solubility limit of DCPA in water (0.5 mg/L; U.S. EPA 1998), but it is not known for certain.

2) The percent inhibition of the solvent control as compared to the negative control was 12.8%, indicating that the solvent may have impacted the growth of *Lemna gibba*.
3) The frond counts of the treatment group was significantly different than the negative control at days 7 and 14 (see appendix 1). The percent inhibition of the frond count in the treatment concentration as compared to the negative control was 31.5%; however, given that due to potential solvent effects, it is not clear if the effect is due to the solvent or the treatment.

4) The percent inhibition of the treatment as compared to the solvent control was 20.6%.

References:

U.S. EPA. 1998. *Reregistration Eligibility Decision (RED): DCPA*. EPA 738-R-98-005. November 1998. Special Review and Reregistration Division, Office of Pesticide Programs. Washington, D.C. U.S.A.

Appendix 1. Statistical Analysis of *Lemna gibba* toxicity data

```
Title:  DCPA Lemna 7 day tox.
File:   DCPALEMNA
     t-Test of Solvent and Blank Controls    Ho: GRP1 Mean = GRP2 Mean
-------------------------------------------------------------------------
GRP1 (Solvent cntl) Mean = 166.0000   Calculated t value =       10.6600
GRP2 (Blank cntl) Mean   = 149.3333   Degrees of freedom =       4
Difference in means      =  16.6667
-------------------------------------------------------------------------
2-sided t value (0.05, 4) = 2.7764**  Significant difference at alpha=0.05
2-sided t value (0.01, 4) = 4.6041**  Significant difference at alpha=0.01
     WARNING: This procedure assumes normality and equal variances!

Title:  DCPA 14-day Lemna tox.
File:          14LMDCPA
     t-Test of Solvent and Blank Controls    Ho: GRP1 Mean = GRP2 Mean
-------------------------------------------------------------------------
GRP1 (Solvent cntl) Mean = 742.0000   Calculated t value =       4.1142
GRP2 (Blank cntl) Mean   = 647.3333   Degrees of freedom =       4
Difference in means      =  94.6667
-------------------------------------------------------------------------
2-sided t value (0.05, 4) = 2.7764**  Significant difference at alpha=0.05
2-sided t value (0.01, 4) = 4.6041    No significant difference at alpha=0.01
     WARNING: This procedure assumes normality and equal variances!

Title:  DCPA Lemna 7 day tox
File:          DCPALEMNA            Transform:            NO TRANSFORMATION
                              ANOVA Table
-------------------------------------------------------------------------
   SOURCE            DF              SS               MS           F
-------------------------------------------------------------------------
   Between            2          4772.2222        2386.1111     271.8354
   Within (Error)     6            52.6667           8.7778
-------------------------------------------------------------------------
   Total              8          4824.8889
-------------------------------------------------------------------------
                                                   (p-value = 0.0000)
   Critical F  = 10.9248  (alpha = 0.01, df = 2,6)
```

```
                = 5.1433  (alpha = 0.05, df = 2,6)
   Since  F > Critical F  REJECT  Ho: All equal (alpha = 0.05)

Title:  DCPA Lemna 7 day tox
File:         DCPALEMNA              Transform:              NO TRANSFORMATION
      Dunnett's Test   -   TABLE 1 OF 2              Ho:Control<Treatment
-------------------------------------------------------------------------------
                              TRANSFORMED    MEAN CALCULATED IN           SIG
GROUP    IDENTIFICATION          MEAN          ORIGINAL UNITS    T STAT  0.05
----- --------------------    -----------    ------------------  ------  ---
  1            neg control    166.0000           166.0000
  2           solv control    149.3333           149.3333         6.8897  *
  3                   11.0    111.0000           111.0000        22.7361  *
-------------------------------------------------------------------------------
Dunnett critical value = 2.3400   (1 Tailed, alpha = 0.05, df = 2,6)

Title:  DCPA Lemna 7 day tox
File:         DCPALEMNA              Transform:              NO TRANSFORMATION
      Dunnett's Test   -   TABLE 2 OF 2              Ho:Control<Treatment
-------------------------------------------------------------------------------
                             NUM OF     MIN SIG DIFF     % OF      DIFFERENCE
GROUP    IDENTIFICATION      REPS     (IN ORIG. UNITS)  CONTROL  FROM CONTROL
----- --------------------  -------  ----------------  -------  ------------
  1            neg control     3
  2           solv control     3            5.6606        3.4       16.6667
  3                   11.0     3            5.6606        3.4       55.0000
-------------------------------------------------------------------------------

Title:  DCPA Lemna 7 day tox
File:         DCPALEMNA              Transform:              NO TRANSFORMATION
                  Tukey Method of Multiple Comparisons
-------------------------------------------------------------------------------
                                                  GROUP
                      TRANSFORMED  ORIGINAL       0 0 0
GROUP  IDENTIFICATION    MEAN        MEAN         3 2 1
----- --------------- -----------  ---------     - - -
  3             11.0    111.0000   111.0000      \
  2      solv control    149.3333   149.3333      * \
  1       neg control    166.0000   166.0000      * * \
-------------------------------------------------------------------------------
* = significant difference (alpha = 0.05)       . = no significant difference
Tukey critical value = 4.3390 (df = 3,6)        s =   8.7778

Title:  DCPA 14-day Lemna
File:          14LMDCPA              Transform:              NO TRANSFORMATION
                              ANOVA Table
-------------------------------------------------------------------------------
   SOURCE             DF              SS                MS            F
-------------------------------------------------------------------------------
   Between             2          80420.6667       40210.3333       52.4787
   Within (Error)      6           4597.3333         766.2222
-------------------------------------------------------------------------------
   Total               8          85018.0000
-------------------------------------------------------------------------------
                                                              (p-value = 0.0002)
   Critical F  = 10.9248  (alpha = 0.01, df = 2,6)
               = 5.1433  (alpha = 0.05, df = 2,6)
   Since  F > Critical F  REJECT  Ho: All equal (alpha = 0.05)
```

Title: DCPA 14-day *Lemna*
File: 14LMDCPA Transform: NO TRANSFORMATION
Dunnett's Test - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG 0.05
1	neg control	742.0000	742.0000		
2	solv control	647.3333	647.3333	4.1886	*
3	11.0	511.6667	511.6667	10.1912	*

Dunnett critical value = 2.3400 (1 Tailed, alpha = 0.05, df = 2,6)

Title: DCPA 14-day *Lemna*
File: 14LMDCPA Transform: NO TRANSFORMATION
Dunnett's Test - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	MIN SIG DIFF (IN ORIG. UNITS)	% OF CONTROL	DIFFERENCE FROM CONTROL
1	neg control	3			
2	solv control	3	52.8868	7.1	94.6667
3	11.0	3	52.8868	7.1	230.3333

Title: DCPA 14-day *Lemna*
File: 14LMDCPA Transform: NO TRANSFORMATION
Tukey Method of Multiple Comparisons

GROUP	IDENTIFICATION	TRANSFORMED MEAN	ORIGINAL MEAN	GROUP 0 0 0 3 2 1
3	11.0	511.6667	511.6667	\
2	solv control	647.3333	647.3333	* \
1	neg control	742.0000	742.0000	* * \

* = significant difference (alpha = 0.05) . = no significant difference
Tukey critical value = 4.3390 (df = 3,6) s = 766.2222

DATA EVALUATION RECORD

1. **CHEMICAL:** Chlorthal Dimethyl.
Shaughnessey No. 078701.

2. **TEST MATERIAL:** DCPA technical (dimethyl tetrachloroterephthalate); CAS No. 1861-32-1; Lot No. 10148/T-170-2; 98.4% active ingredient; a tan powder.

3. **STUDY TYPE:** 122-2. Growth and Reproduction of Aquatic Plants - Tier 1. Species Tested: *Lemna gibba*.

4. **CITATION:** Hughes, J.S. and P.H. Balcom. 1993. The Toxicity of DCPA Technical to *Lemna gibba* G3. Laboratory Project ID No. B038-033-4. Conducted by Malcolm Pirnie, Inc., Tarrytown, NY. Submitted by ISK Biotech Corporation, Mentor, OH. EPA MRID No. 428361-01.

5. **REVIEWED BY:**

Michael W. Davy **Signature:** Michael Davy
Agronomist
Ecological Effects Branch **Date:** 3-25-94
Environmental Fate and Effects Division

6. **APPROVED BY:**

Daniel Rieder **Signature:** Daniel Rieder
Section Head
Ecological Effects Branch **Date:** 5-12-94
Environmental Fate and Effects Division

7. **CONCLUSIONS:** This study is scientifically sound and meets the guideline requirements for a Tier 1 non-target aquatic plant study using *Lemna gibba* G3. Based on the nominal concentrations, the EC_{50}> 11.0 ppm during the 14-day test period.

8. **RECOMMENDATIONS:** N/A.

9. **BACKGROUND:** Reregistration data

10. **DISCUSSION OF INDIVIDUAL TESTS:** N/A.

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11. MATERIALS AND METHODS:

A. Test Species: *Lemna gibba* G3 used in the test came from laboratory stock cultures originally obtained from the USDA, Beltsville, MD. Stock cultures were maintained in synthetic twenty-strength algal assay procedure nutrient medium (20X-AAP) under 4.2-5.8 klux illumination, at a temperature of 25 ±2°C. Transfers were made regularly to provide 6- to 11-day old cultures. The culture used as inoculum in this test had been transferred to fresh medium eleven days before test initiation.

B. Test System: All glassware was cleaned and autoclaved before use. Test vessels used were 500-ml Erlenmeyer flasks fitted with foam stoppers which permitted gas exchange. The test medium was the same as that used for culturing with the pH adjusted to 7.5 ±0.1. The medium was filter sterilized (0.22 μm) prior to inoculation.

The test vessels were kept in an incubator under environmental conditions like those employed in culturing with continuous warm-white fluorescent illumination.

A 22 mg active ingredient (ai)/ml stock solution was prepared by dissolving 559.1 mg of the test material in N,N-dimethylformamide (DMF) and diluting to a final volume of 25 ml. The test solution was prepared by adding 0.5 ml of the stock to 1 l of nutrient medium.

C. Dosage: Fourteen-day growth and reproduction test. One nominal concentration of 11 mg ai/l was selected for the test. A solvent control (0.5 ml DMF/l of nutrient solution) and a medium control were also prepared. The maximum labeled application rate for DCPA was reported to be 15 lb ai/acre. This is equivalent to 11.0 mg ai/l if applied to a 15-cm water column.

D. Test Design: Two-hundred ml of the appropriate test or control solution were placed into each of three replicate flasks for each treatment and control.

The plants were aseptically added to the nutrient medium. An inoculum of *Lemna gibba* consisted of three plants per flask, each with four fronds. The flasks were randomly repositioned each working day to minimize spatial differences in the incubator. Frond counts

were performed on test days 3, 5, 7, 10, 12, and 14. Every frond that visibly projected beyond the edge of the parent frond was counted.

Temperature in the incubator was measured manually daily and automatically continuously. The pH was measured at test initiation and termination. Analytical measurements of the test material in the treatment solution were not performed.

E. **Statistics:** Percentage inhibition was determined by comparison of the terminal treatment frond number to that of the solvent control. If the treatment resulted in inhibition of greater than or equal to 50%, then Tier 2 testing is indicated.

12. **REPORTED RESULTS:** Throughout the test, the treatment solution appeared cloudy with white particulates in suspension. The treatment concentration (11 mg ai/l) was 22 times greater than the reported maximum water solubility of DCPA (0.5 mg ai/l).

Frond counts and percentage inhibition after 14 days are given in Tables 3 and 4 (attached). Percentage frond inhibition was 21.4% in comparison to the solvent control. It was also noted that treated plants tended to have a larger number of fronds per plant, but the size of the fronds was reduced.

The pH ranged from 7.87 to 7.90 in the test solutions at study initiation. The pH values on day 14 ranged from 9.13 to 9.66.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** The authors concluded that Tier 2 testing was not required due to less than 50% inhibition observed at the tested concentration of 11 mg ai/l.

Good Laboratory Practice and Quality Assurance statements were included in the report indicating compliance with EPA Good Laboratory Practice Standards, 40 CFR Part 160.

14. **REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:**

A. **Test Procedure:** The test procedure and the report were generally in accordance with the SEP and Subdivision J guidelines, except for the following deviations:

The results of the temperature measurements were not reported.

The light intensity (4.2-5.8 klux) was occasionally lower or higher than recommended (5 klux).

Three plants with four fronds each were used as the inoculum rather than the recommended five plants with three fronds each.

B. **Statistical Analysis:** The reviewer used a t-test to determine if a significant reduction in frond number had occurred during the test between the two controls and between the solvent control and treatment. The results of the analysis indicated that DCPA technical at a nominal concentration of 11 mg ai/l significantly reduced the frond number of treated plants over a 14 day period (see attached printouts).

C. **Discussion/Results:** The treatment solution appeared cloudy throughout the test. The reviewer has no doubt that the material was present at its maximum solubility (0.5 mg ai/l).

This study is scientifically sound and meets the guideline requirements for a Tier 1 non-target aquatic plant study. Based on the nominal concentrations, the EC_{50}> 11.0 ppm during the 14-day test period.

D. **Adequacy of the Study:**

(1) Classification: Core

(2) Rationale: N/A

(3) Repairability: N/A

15. **COMPLETION OF ONE-LINER:** Yes

DATA EVALUATION RECORD

1. **CHEMICAL:** Chlorthal Dimethyl.
Shaughnessey No. 078701.

2. **TEST MATERIAL:** DCPA technical (dimethyl tetrachloroterephthalate); CAS No. 1861-32-1; Lot No. 10148/T-170-2; 98.4% active ingredient; a tan powder.

3. **STUDY TYPE:** 122-2. Growth and Reproduction of Aquatic Plants - Tier 1. Species Tested: *Lemna gibba*.

4. **CITATION:** Hughes, J.S. and P.H. Balcom. 1993. The Toxicity of DCPA Technical to *Lemna gibba* G3. Laboratory Project ID No. B038-033-4. Conducted by Malcolm Pirnie, Inc., Tarrytown, NY. Submitted by ISK Biotech Corporation, Mentor, OH. EPA MRID No. 428361-01.

5. **REVIEWED BY:**

Mark A. Mossler, M.S.
Agronomist
KBN Engineering and
Applied Sciences, Inc.

Signature: Mark A. Mossler
Date: 9/27/93

6. **APPROVED BY:**

Pim Kosalwat, Ph.D.
Senior Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: P. Kosalwat
Date: 9/27/93

~~Henry T. Craven~~, M.S.
Supervisor, EEB/EFED
USEPA

Signature:
Date: 3 23 94

7. **CONCLUSIONS:** This study is scientifically sound but does not meet the guideline requirements for a Tier 1 non-target aquatic plant study. The actual concentration of DCPA technical in solution was not determined. Based on the maximum water solubility of the test material (0.5 mg ai/l), a significant reduction (21%) in frond number occurred during the 14-day test period.

8. **RECOMMENDATIONS:** N/A.

9. **BACKGROUND:**

10. **DISCUSSION OF INDIVIDUAL TESTS:** N/A.

DCPA Technical: *Lemna gibba* Toxicity Test

Table 3. Frond counts during test

Nominal Concentration, mg/L		Day 3 3-8-93	Day 5 3-10-93	Day 7 3-12-93	Day 10 3-15-93	Day 12 3-17-93	Day 14 3-19-93
No-treatment	A	43	86	167	396	569	708
Control	B	40	90	168	376	501	742
	C	42	94	163	359	510	776
	Mean	42	90	166	377	527	742
	SD[1]	2	4	3	19	37	34
	Var[2]	2	16	7	343	1364	1156
Solvent	A	40	85	149	308	477	632
Control	B	40	86	150	314	470	639
	C	34	77	149	309	509	671
	Mean	38	83	149	310	485	647
	SD	3	5	1	3	21	21
	Var	12	24	0	10	432	432
11.0	A	32	69	113	257	333	492
	B	31	65	114	244	337	542
	C	28	64	106	239	350	501
	Mean	30	66	111	247	340	512
	SD	2	3	4	9	9	27
	Var	4	7	19	86	79	710

[1] SD = standard deviation

[2] Var = variance

DCPA Technical: *Lemna gibba* Toxicity Test

Table 4. Percent inhibition, relative to solvent control, based upon mean frond counts on day 14

Nominal Concentration, mg/L	Mean Frond Counts on day 14	Percent Inhibition
Solvent Control	647	---
11.0	512	21.4

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                    º STUDENT'S T-TEST (two-tailed) º
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        Enter the name of the DATAFILE you wish to analyze:  lem
      (Press RETURN if you wish to skip directly to T evaluation)

   What are the SAMPLE NUMBERS of the 2 variables you want to compare?
                1 `c'                          2 `sc'
    Means =        742                            647.25
Variances =        770.6665                       288.2501

        Are these INDEPENDENT or PAIRED samples? (I or P)    i

          T =  5.823423                                df =  6
                                 p =  1.127601E-03
       The MEANS of these 2 samples are significantly different.

The confidence limits on the DIFFERENCE between the means of these samples
can be calculated as:
                          94.75 +/- T(6) *  16.2705


             Do you want another T-TEST using this datafile?
```

```
                    É͸͸͸͸͸͸͸͸͸͸͸͸͸͸͸͸͸͸͸͸͸͸͸͸͸͸»
                    º STUDENT'S T-TEST (two-tailed) º
                    È͸͸͸͸͸͸͸͸͸͸͸͸͸͸͸͸͸͸͸͸͸͸͸͸͸͸¼

        Enter the name of the DATAFILE you wish to analyze:  lem
      (Press RETURN if you wish to skip directly to T evaluation)

   What are the SAMPLE NUMBERS of the 2 variables you want to compare?
                1 `solcont'                    2 `trt'
    Means =        647.25                         511.75
Variances =        288.2501                       473.5834

         Are these INDEPENDENT or PAIRED samples? (I or P)    i

           T =  9.818368                                 df =  6
                                  p =  6.431341E-05
       The MEANS of these 2 samples are significantly different.

The confidence limits on the DIFFERENCE between the means of these samples
can be calculated as:
                          135.5 +/- T(6) *  13.80066



              Do you want another T-TEST using this datafile?
```